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# LARVAL SETTLING BEHAVIOR IN *CYANEA CAPILLATA* (CNIDARIA: SCYPHOZOA)

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The interactions between organisms and their environment are complex and reflect the adaptive response to a particular set of conditions by each stage in the life history. This is especially conspicuous in species with dissimilar life history stages, each with different demands on the environment. The success of such species requires that the adaptations of the immature forms lead to maturation by the adult in an environment suitable for successful reproduction. This can be a stringent requirement in species with a motile larva whose pattern of settlement effects the distribution of a sessile or sedentary adult; larval behavior adapted for the selection of substrate thus can maximize survival of the attached reproductive stage. This life history sequence, and the potential capacity for substrate selection, is characteristic of many marine invertebrates.

Numerous workers have analyzed marine larval behavior in the higher phyla, and they show that larvae recognize and select specific substrates with which their sedentary adult stages are associated. The most intensive studies deal with the larvae of polychaetous annelids, the pelecypod molluscs, and Cirripedia (Arthropoda). Recent reviews (Newell, 1970; Meadows and Campbell, 1972; Crisp, 1974) summarize this work, and also report studies on the Ectoprocta and Urochordata. These larvae respond to light, gravity, or current in a manner which leads them to the general vicinity where attachment can occur. Once in contact with the substrate, the larva reacts to surface texture and its chemistry. These characteristics are sorted by the larva during a behavioral sequence which leads to the selection on a firm substrate of a specific site for attachment; this sequence is similar among the diverse species studied (*e.g.*, Reese, 1964). Crisp (1974, p. 196) divides this searching behavior into three phases, the last of which is a period of "inspection", a minute examination of the substrate surface; if the stimuli received by the larva during inspection are appropriate, attachment follows. The knowledge of larval behavior in the higher phyla is sufficient to allow the formation of theoretical models of habitat selection (*e.g.*, Doyle, 1975).

The extent to which the larvae of lower phyla select specific sites for attachment requires investigation to determine whether these fit the pattern of behavior shown by more complexly organized larvae typical of the higher phyla. The planula larva of the Cnidaria, a motile (stereo-) gastrula (*e.g.*, Mergner, 1971; Campbell, 1974), is morphologically simple compared to the comparable stage in the phyla most studied. The purpose of this paper is to determine if the planula stage of the Scyphozoa exhibits behavioral responses which can account for the distribution of its sedentary stage (the scyphistoma) in the field. The scyphistoma is usually found suspended upside-down from a hard surface in a shaded location (Wilson, 1952; Hardy, 1958; Fraser, 1962; Cargo and Schultz, 1966, 1967; personal observation), the position usually selected by marine larvae (*e.g.*, Crisp, 1974).

The response of the planulae of *Cyanea capillata* (Linneaus) to the direction of gravity and to light and their behavior on hard substrate of different texture or contour is examined in this paper. The effects that conditions associated with low levels of oxygen might have on their attachment behavior is considered, because scyphistomae are commonly found near the bottom of shallow estuaries (*e.g.*, Thiel, 1962) where these conditions must often be encountered by planulae. The settlement of *Cyanea* planulae is frequently accompanied by encystment (*e.g.*, MacMurrich, 1891; Hyde, 1894; Hargitt, 1902; Hargitt and Hargitt, 1910; Rees, 1957; Cargo and Schultz, 1967; Widersten, 1968; Cargo, 1975); the possible adaptive significance of this phenomenon is briefly discussed.

## Materials and Methods

Planulae were obtained from gravid specimens of *C. capillata* (7 to 15 cm diameter) collected from Smith's Cove, Niantic, Connecticut, during the months of May and June, 1973 and 1974. Marshall (1960) provides a hydrographic description of this area.

### *General experimental conditions*

Fresh, filtered sea water (salinity, 24‰; initial temperature, 15° C) was used in all experiments; these conditions correspond to those at the time the planulae were collected. The experiments were set up within three hours after obtaining the planulae and were run at room temperature (19–21° C) with one exception, noted below; the illumination was flourescent and constant.

### *Response to the direction of gravity*

A 15-gallon aquarium, its sides screened with black construction paper, was used during the measurement of the response of planulae to the direction of gravity. Into this aquarium was placed an apparatus made of one-eighth inch diameter glass rods to which 2 $cm^2$ plastic coverslips were fastened with silicon-rubber cement; these rods were of different lengths and were attached to a lucite platform which rested on the aquarium frame. Thus, the coverslips were suspended at different heights above the aquarium bottom. Each height was replicated five times in a $5 \times 5$ Latin square, of which there were two: a lower set at heights of 5, 10, 20, 40, and 80 mm; and a higher set at heights of 100, 150, 200, 250, and 275 mm. Beginning at the time the planulae were added, observations were made at least twice within each 24 hour period for six days to determine the number of planulae swimming at different heights in a water column one cm wide, extending from the front to the back of the aquarium. When the planulae had attached, the numbers on the coverslips, the aquarium bottom and walls, and on the underside of the water surface (the hyponeuston) were recorded. The experiment was repeated with the positions of the low and high sets of coverslips reversed 180° relative to the aquarium.

### *Orientation of planulae at time of attachment and effect of surface texture*

The surface upon which planulae attach, beneath or upon the uppermost surface of an object, and whether planulae distinguish between different surface textures were simultaneously determined. Planulae were added to a 20 cm diameter bowl in the bottom of which were arranged 16 plastic coverslips held 3 mm from the bottom by split-shot clipped to their corners. One half of the coverslips, on both the upper and the lower surfaces, was roughened with sandpaper (100 grit) to effect four treatments: R/R, R/S, S/R, S/S, where R represents rough, S represents smooth, and the letter to the left of the slash represents the upper surface. These treatments were arranged in a 4 × 4 Latin square. Two experiments of this design were run at the same time. The numbers of planulae attached to each of the coverslip surfaces, as well as the number which settled on the upper and the lower hemispheres of the split-shot, were recorded after they had attached.

### *Response of planulae to surface contour*

The response to surface contour was determined by exposing planulae to 2 $cm^2$ plastic coverslips, the lower surfaces of which were scratched with a dissecting needle to produce shallow grooves in a grid-shaped pattern. The different coverslips were distinguished by the total length of grooves on their surface. There were two treatments: one with 10 linear cm and another with 49 linear cm of grooves; unmarked (0 cm) coverslips served as controls. The coverslips were arranged in a 3 × 3 Latin square, and were supported 3 mm above the bottom of an 11 cm diameter finger bowl.

### *Response of planulae to light*

*Shaded attachment sites.* To establish whether planulae discriminate between shaded and nonshaded sites at the time of attachment, a 4 × 4 Latin square arrangement of supported coverslips were placed on the bottom of a 20 cm diameter bowl. One half of these coverslips were painted black on the upper surface, and one half of these coverslips were roughened on the lower surface. The four treatments were S/S, S/R, B/S, and B/R, where B represents the painted upper surface; the other letters have the same meaning as noted previously. A 30 cm tall cylinder of black paper, open at the top, was fit around the dish after the planulae had been added to increase the contrast in the level of illumination beneath the painted and the unpainted coverslips.

*Direction of light.* Planulae were exposed to four patterns of illumination to determine the effect that the direction of light might have upon their orientation at the time of attachment. The planulae in treatment i were put into 5 cm diameter (× 2.5 cm tall) covered stendor dishes and maintained in complete darkness. Black construction paper was fitted around the same sized dishes to make treatments ii, iii, and iv; a cylinder of black paper masked the dish sides in these treatments. Black paper covered the dish bottom (treatment ii) and the dish top (treatment iii) to allow light to enter from only one direction, from above and from below, respectively; in treatment iv, neither the top nor the bottom of the dishes was covered permitting illumination from both above and below the container. The dishes for treatments iii and iv were set on transparent glass plates to permit illumination from below. Ten replicates for each treatment were set up in random sequence with each replicate containing 20 planulae. The treatments were positioned randomly in two constant temperature cabinets: one cabinet was maintained without light (treatment i) and the other cabinet was evenly illuminated with directional light effected by black paper (treatments ii, iii, and iv). The temperature averaged 17.48 ± 0.147° C and 17.51 ± 0.170° C, respectively, for the two cabinets. The position of the attached planulae was determined on the 27th day.

### *Response of planulae to anaerobic conditions*

Three air-tight containers to which planulae had been added were used to assess the effects which a lowered concentration of oxygen might have upon their attachment behavior: one contained deteriorating tissues from a moribund medusa and five supported coverslips; the other two contained fresh, filtered sea water and six supported coverslips, each. In one of the latter, air was bubbled, and in the other, nitrogen. After a period of two hours, the tubing was adjusted so that the water was no longer disturbed, but so that the volume above the water surface remained either pure air or pure nitrogen; an aperture in each container allowed introduced gas to escape. At the end of two days, the distribution of the planulae attached to upper and lower surfaces of the coverslips, to the bottom of the container beneath the coverslips, and to the hyponeuston above the coverslips was determined.

## RESULTS

### *General observations*

The mature planula is about 185 μ long, wider and blunt at the anterior end, biconcave in cross-section (Figure 1A), and yellow in color. Prior to settlement, the anterior-posterior axis becomes shortened and the cross-section becomes more circular. In this form, the planula appears to actively inspect (*sensu* Crisp, 1974) any surface with the anterior end by rotating counter-clockwise (viewed from the posterior pole) around its longer axis. If this surface is not suitable, the planula leaves it, swims for a variable distance, and then approaches the surface to inspect another site. Upon contact with a satisfactory site the planula becomes affixed and assumes a pyramidal shape (Figure 1B), which during three hours at 20° C becomes simultaneously reduced in height and increased in diameter (Figure 1C and 1D). The nipple at the apex disappears and a flange about 18 μ wide appears after six to ten hours (Figure 1E). This is the encysted planula from which, after a variable period of time, the polyp begins to emerge (Figure 1F). The encysted stage is firmly attached to the substrate, is an objective criterion for settlement, and is the stage enumerated in the experimental results which follow.

### *Analysis of the experimental results*

Most of the data require transformation before analysis. Those data for which a log transformation is utilized are reported as antilogs (with 0.95 confidence limits). An angular transformation is used for proportional data (except for the observations on mortality); these results are reported as $P = 100[\Theta]^2$, (± s.d.). Bartlett's test for homogeneity of variances is nonsignificant (at least, $P > 0.10$) for all but one comparison which is mentioned in the appropriate place below.

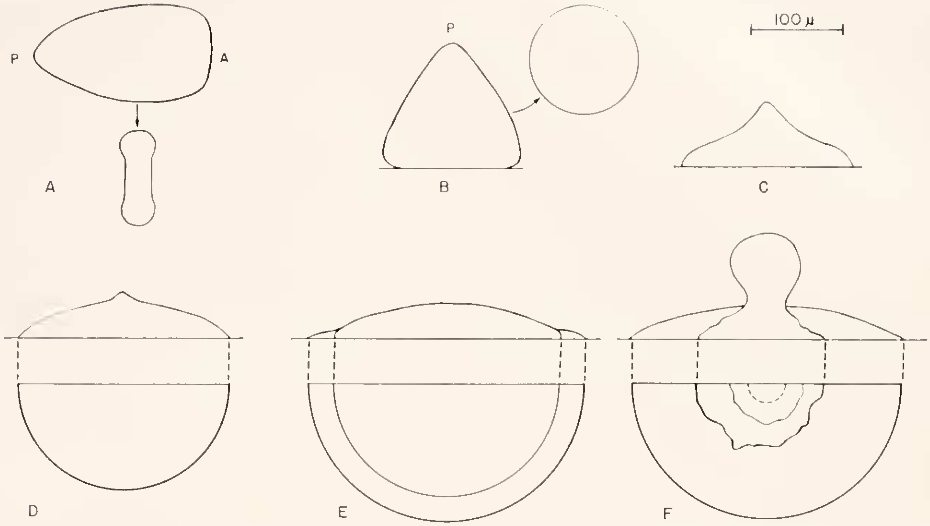

FIGURE 1. The early stages in the life history of *Cyanea capillata:* A, planula (view from above, upper diagram; cross-section, lower diagram); B, initial attachment (side view, left diagram; cross-section, right diagram); C, flattening of attached planula (side view); D and E, later stages and cyst formation (side view, upper diagram; view from above—one half of cyst shown, lower diagram); and F, excystment (views same as in D and E). A represents anterior; P, posterior (see text for details).

Tests of significance are based on the analysis of variance, model I. The row and column effects are nonsignificant in the experiments where a Latin square design is used.

### *Response to the direction of gravity*

Planulae put into the aquarium accumulate on the bottom after only 30 minutes; the planulae move about slowly and concentrate in the lowest positions on the slate bottom until the 45th hour, filling in all grooves and depressions, which as a consequence appear as yellow lines and patches, respectively. Between 45 and 52 hours, their concentration in these surface irregularities are noticeably reduced as the planulae become more active and begin to swim less than five mm above the bottom. Planulae are conspicuous in the water column only after 69 hours at which time encystment begins on the coverslips suspended five mm above the bottom. At subsequent observations (between 72 and 144 hours), planulae remain in the water column. Throughout the latter period, concentrations of planulae are more or less stable up to a height of 8 to 12 cm; the maximum height attained by some planulae above this level (up to the surface of the water in the tank—28 cm) varies erratically until the last of these observations made on the sixth day. All planulae encyst by the ninth day.

The effect of this behavior on the position of attachment by planulae is shown by the distribution of their cysts on surfaces in the aquarium. In the two trials of this experiment, only 5.8% (1,518 cysts) and 17.3% (192 cysts) are attached to the aquarium bottom, respectively; the balance of the cysts are attached either to the suspended coverslips or to the hyponeuston, except for 0.6% (162 cysts) which are found on the aquarium walls in the first trial. The number of planulae which encyst on the coverslips—14,392 and 573, respectively—decreases linearly with distance from the bottom; this relationship is described by the following two equations: $\text{Log } \hat{Y} = \text{Log } 6306.2857 - \text{Log } 16.0844X$ and $\text{Log } \hat{Y} = \text{Log } 186.7542 - \text{Log } 7.6325X$, respectively. The regressions are significant ($P < 0.001$) for both sets of data, and the deviations from regression are nonsignificant ($P > 0.75$) for the former, but are significant ($P < 0.001$) for the latter, due to an unexplained heterogeneity.

The planulae apparently are initially geopositive and sometime between the 52nd and the 69th hour become geonegative prior to attachment; the planulae remain in contact with the bottom until between the 45th and 52nd hour. The linear gradient in density of attachment on suspended coverslips show that planulae attach most frequently close to, but not on, the bottom.

### *Orientation of planulae at time of attachment and effect of surface texture*

The average number (with 0.95 confidence limits) and the number of planulae encysting on upper and lower surfaces of supported coverslips of different textures are shown in Table I; "coverslips (I)" and "coverslips (II)" show data from replicate experiments. Planulae encyst in greatest numbers beneath supported coverslips ($P < 0.001$) and upon a surface which is roughened ($0.025 < P < 0.05$).

The distribution of the planulae which encyst in positions other than on the coverslips was determined for those in one replicate experiment (I). These data for the planulae attaching to split-shot are shown in Table I: 84.6% (n = 7474) of the cysts are attached to the lower surface (hemisphere) of the shot ($P < 0.001$); but there is no difference ($P > 0.75$) in the number of cysts on the shot with respect to the rugosity of the coverslips that they support (columns, Table I). Note that

TABLE I

*Orientation of planulae of* Cyanea capillata *at time of attachment to coverslips and to split-shot and their response to surface texture.*

| Orientation (surface of coverslip or split-shot) | | Texture (coverslip) | | | | Total | |
|---|---|---|---|---|---|---|---|
| | | Rough | | Smooth | | | |
| | | Number attached | Mean (0.95 confidence limits) | Number attached | Mean (0.95 confidence limits) | Number attached | Mean (0.95 confidence limits) |
| Upper | Coverslips (I) | 373 | 39 (22, 68) | 196 | 20 (12, 35) | 569 | 28 (19, 41) |
| | Coverslips (II) | 621 | 73 (53, 101) | 420 | 48 (33, 72) | 1,041 | 59 (47, 76) |
| | Split-shot | 662 | 68 (39, 119) | 674 | 72 (44, 118) | 1,336 | 70 (51, 97) |
| Lower | Coverslips (I) | 8573 | 752 (350, 1618) | 4614 | 465 (249, 867) | 13,187 | 591 (377, 927)*** |
| | Coverslips (II) | 9036 | 975 (593, 1602) | 6307 | 673 (394, 1151) | 15,343 | 810 (580, 1131)*** |
| | Split-shot | 3562 | 416 (299, 579) | 3912 | 435 (275, 687) | 7,474 | 425 (332, 544)*** |
| Total | Coverslips (I) | 8946 | 171 (69, 426)* | 4810 | 97 (38, 247) | 13,756 | |
| | Coverslips (II) | 9657 | 267 (125, 569)* | 6727 | 180 (83, 393) | 16,384 | |
| | Split-shot | 4224 | 169 (95, 298) n.s. | 4586 | 177 (99, 314) | 8,810 | |

* $0.025 < P < 0.05$.
*** $P < 0.001$.
n.s. $P > 0.75$.

all of the shots possess the same surface characteristics; the data are indicated in separate columns to show the type of coverslip which the shot support. This difference between the outcome for split-shot and that for coverslip texture, above, strengthens the conclusion that roughened substrates attract greater numbers of planulae. The number of planulae attaching to the bottom, to the hyponeuston, or those not encysting at the time the counts were made (three days after the experiment began) total 352 (2.5%).

The basic design of the experiment to determine the response of planulae to "shaded attachment sites" is similar to that used in the experiment just described. The results of that experiment, in the present context are the same with regard to orientation at time of attachment, but differ in one respect in the response of planulae to texture. For example, 82.0% (n = 14,753) of the planulae attach to the lower surface of coverslips, and 88.9% (n = 13,112) of these encyst beneath coverslips with a rough lower surface. However, of the 18% (3223) of the planulae which settle on the upper surface, an average of 346 (251, 477) planulae attach to each coverslip which had the upper surface painted, compared to an average of 34 (24, 47) planulae attaching to the unpainted (smooth) upper surface of coverslips; this is a 10-fold difference. In contrast, the ratio of the average number of planulae encysting on the rough upper surface to those attaching to smooth (upper surface) coverslips shown at the top of Table I for this experiment are 1.95 (39:20) and 1.52 (73:48), respectively, for the two trials. This suggests that planulae respond to the fine texture (pigment granules) of the painted surface more strongly than to a course roughness (sandpapered surface).

### *Response of planulae to surface contour*

There is a positive linear relationship between the length of the grooves scratched on the lower surface of coverslips and the number of planulae which attach to them during a 24 hour period. On unetched (control) coverslips, 238 ± 48 planulae encyst; on coverslips with 10 linear cm of scratched surface, 554 ± 44 planulae attach; and on those with 49 cm of grooves, 1180 ± 103 planulae settle.

### *Response of planulae to light*

*Shaded attachment sites.* The distribution of planulae encysting after nine hours beneath opaque (painted) coverslips and beneath transparent coverslips does not differ ($P > 0.75$). The average number (and 0.95 confidence limits) on the undersurface of each opaque coverslip was 512 (198, 1324) and underneath each transparent coverslip, 444 (114, 1719). No free-swimming planulae remained at the time counts were made.

*Direction of light.* The effect of different patterns of light on the orientation at time of attachment and the mortality of planulae is shown in Table II. The angular transformation of the data reduces, but does not eliminate, the heterogeneity of variances. An approximate test for these data show that the proportion of planulae which encyst upside-down (on the hyponeuston) is highest ($P < 0.001$) if the light is projected from beneath their container. An *a posteriori* test shows no difference in the proportion which attach upside-down among the remaining three treatments. These results suggest that the planulae are photo-negative at time of attachment, settling with greatest frequency on a surface opposite the light source, but since the absolute differences among the remaining treatments are slight this response to light appears to be weak relative to their orientation to direction of gravity.

Mortality of planulae is greatest ($P < 0.001$) for those maintained in total darkness ("none", in Table II); there is no difference in mortality among treatments which receive light ($0.10 < P < 0.25$).

Fifteen planulae (1.97%) were unattached when the experiment was terminated on the 27th day; the distribution of these among the treatments can be determined from the data provided in Table II. Neither their attachment nor their death would change the outcome above.

### *Response of planulae to anaerobic conditions*

The orientation at the time of attachment by planulae both on coverslips and in different positions in their container under anaerobic conditions is shown in Table III (no planulae attached to the walls of their container). The initial number (not known) of planulae used in each treatment definitely was more similar than shown in Table III. The differences in the number of planulae among the three treatments possibly are, on the one hand, due to an increase in planulae in the treatment to which organic matter was added because of completed embryogeny from blastulae associated with the gonadal tissue; and on the other, due to higher mortality in the treatment with nitrogen.

Both sets of data (coverslips and container) indicate that relatively few planulae attach rightside-up when exposed to air, and that proportionately more planulae attach rightside-up in the absence of oxygen (nitrogen) than in the treatment with

TABLE II

*Effect of different illumination on the orientation at time of attachment and the mortality of planulae of* Cyanea capillata.

| Position of light source (number of planulae) | Attachment | | | Mortality | |
|---|---|---|---|---|---|
| | Site | Number attached | Percent surviving planulae attached upside-down (±s.d.) | Number | Percent (±s.d.) |
| None<br>(200) | Hyponeuston<br>Dish bottom | 129<br>19 | 91.0 ± 6.07 | 52 | 26.0 ± 9.37*** |
| Above culture<br>(200) | Hyponeuston<br>Dish bottom | 154<br>18 | 92.9 ± 3.46 | 18 | 9.0 ± 6.15 |
| Below culture<br>(200) | Hyponeuston<br>Dish bottom | 171<br>1 | 99.9 ± 0.56*** | 26 | 13.0 ± 8.23 |
| Above and below culture<br>(160)† | Hyponeuston<br>Dish bottom | 127<br>15 | 92.2 ± 3.89 | 15 | 8.0 ± 6.75 |

† Two replicates with 20 planulae each were lost.
*** $P < 0.001$.

TABLE III

*The orientation at time of attachment by planulae of* Cyanea capillata *in response to anaerobic conditions.*

| Treatment | Site of attachment | Number of planulae attached | Percent of planulae attached rightside-up (± s.d.) |
|---|---|---|---|
| Air (control) | Coverslip | | |
| | Upper | 51 | 6.3 ± 0.71** |
| | Lower | 772 | |
| | Container | | |
| | Hyponeuston | 404 | 4.6 ± 9.63*** |
| | Bottom | 11 | |
| Organic material† | Coverslip | | |
| | Upper | 133 | 8.1 ± 0.55 |
| | Lower | 1888 | |
| | Container | | |
| | Hyponeuston | 123 | 59.8 ± 11.29 |
| | Bottom | 168 | |
| Nitrogen | Coverslip | | |
| | Upper | 148 | 30.3 ± 1.97*** |
| | Lower | 331 | |
| | Container | | |
| | Hyponeuston | 17 | 77.1 ± 14.40* |
| | Bottom | 72 | |

† Organic material represents pieces of oral folds, gonadal tissue, and tentacles from a medusa.
* $0.025 < P < 0.05$; ** $0.001 < P < 0.005$; *** $P < 0.001$.

organic material added; in the latter, the oxygen content could be expected to be at an intermediate level. An *a posteriori* comparison shows that the difference in the proportion rightside-up on coverslips between air and organic material is nonsignificant.

Three hours after this experiment was set up, many planulae were observed to be active and swimming throughout the water volume in the treatment to which organic material was added; very few planulae were seen in the treatment exposed to nitrogen. No planulae were observed in the control which means that they must have been on the bottom (the refraction at the bottom of the glass dishes prevented observation from the side, and the covers were translucent). The possible significance both for this difference in activity, and for the results showing orientation of planulae at time of attachment in Table III, are made more clear by a fortuitous observation during the first experiment ("response to the direction of gravity") described in this section.

In the two trials of the first experiment, few planulae attach rightside-up on the bottom of the aquarium. In contrast, during another trial (not reported) intended to yield identical data, the results are different: 61.6% (n = 19,296) of the planulae encyst rightside-up; and prior to encystment they swim throughout the water column without an initial settling, *en masse,* to the bottom. However, in setting up this trial the expedient was taken neither to filter nor to aerate the water used before adding the planulae. Examination of this water to determine the vertical distribution of the planulae showed it to contain a dense population of ciliates, in excess of $10^5$ cells/ml (estimate made later). Such large populations of protozoa could alter the dissolved gas content of the medium.

Thus, planulae might become more active and swim away from the bottom under conditions associated with low oxygen; under these conditions in the laboratory, attachment is most frequently rightside-up.

These laboratory experiments are not considered to be a substitute either for experimentation or for observation in the field; however, these data do provide the basis for a reasonable hypothesis which can focus field investigation.

## Discussion

These data suggest the sequence of events which may occur in the field during the early larval period of *C. capillata* (see Figure 2). The planulae, upon leaving the oral folds of the parent medusa, descend quickly to the bottom (Figure 2A) where their continuing movements take them to the lowest level within the area of their initial settling (Figure 2B). The consequences of this are two-fold: first, the planulae are most likely to encounter anaerobic conditions at the water-sediment interface (see below) which stimulates them to swim upwards (Figure 2C); and second, swimming upwards from the lowest position increases the probability of contact with the undersurface of an object. The latter possibly is enhanced by a weak photonegative response (Figure 2D) which inhibits the planulae from swim-

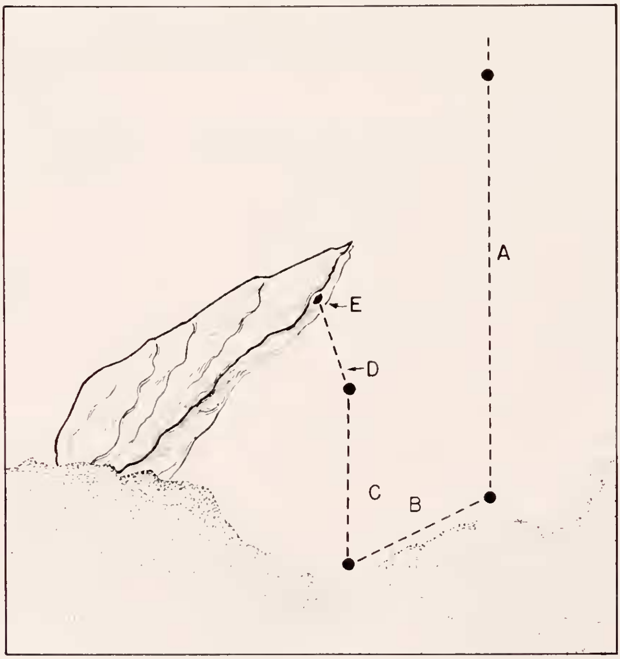

Figure 2. The pattern of behavior shown by the planula of *Cyanea capillata* in the laboratory extrapolated to conditions in the field (see text for details).

ming around the margin of an object. Attachment to the substrate, by encystment, follows the selection of an appropriately rough site (Figure 2E). This sequence leads to the attachment by planulae upside-down.

The attachment upside-down by planulae of *Cyanea* has been noted by others. Perez (1920–21) observed the attachment of *Cyanea* planulae to the undersurface of pieces of mussel shell placed with them in culture dishes, and Phillips (1972) mentions similar observations; I have obtained the same results on shells of *Mytilus*, *Modiolus*, and *Mercenaria*. However, a number of authors remark upon the settlement of scyphozoan planulae rightside-up on the bottom of their containers (Sars, 1841; Hargitt and Hargitt, 1910; Perez, 1920–21), and others imply that this is the usual orientation at the time of attachment (Agassiz, 1862; MacMurrich, 1891; Cargo, 1975). This study shows that significantly more planulae attach upside-down in fresh sea water than in the opposite position; this is consistent with field observations on the position occupied by scyphistomae.

The polyps of *Aurelia* are most frequently found attached upside-down (Wilson, 1952; Hardy, 1958; Fraser, 1962), and the photographs in Buchsbaum (1972) show this orientation, as does the picture of strobilating polyps of *C. capillata* [?] in Jägersten (1960). I have seen scyphistomae (sp.?) attached upside-down on rocks collected from the northwest Pacific. This is also the usual orientation for the scyphistoma of *Chrysaora quinquecirrha* on oyster shells in Chesapeake Bay (Cargo and Schultz, 1966, 1967; Cones and Haven, 1969). In addition, Cargo and Schultz (1967) report field experiments which show that more planulae of *C. quinquecirrha* attach to the lower surface of suspended glass plates than to the upper surface, but regard their data to be insufficient to reach a definite conclusion.

The orientation to, and the selection of, specific sites by marine larvae represents their final response to a series of environmental cues. Initially, most larvae in the higher phyla are negatively geotactic and/or positively phototactic upon release; reversal of these taxes, associated with age and/or developmental stage (Reese, 1964; Newell, 1970; Meadows and Campbell, 1972; Crisp, 1974), occurs prior to attachment. This pattern of response to light (and possibly also to gravity) is apparently true for madreporarian planulae (*e.g.*, Yonge, 1963, 1968).

With respect to light, Williams (1965) shows that the planulae of *Clava squamata* (Hydrozoa) are initially photopositive, becoming photonegative with age, especially if in contact with suitable algal substrate; at time of settlement, the planulae of *Hydractinia echinata* (Hydrozoa) are photopositive (Teitelbaum, 1966). On the other hand, at least the early planulae of *Cyanea* appear to be indifferent to light; many hours of observation with both flourescent and incandescent light at different intensities and from different angles give no indication that light influences their early behavior. Cargo and Schultz (1966) indicate a lack of photosensitivity in the planulae of *C. quinquecirrha*, and the planulae of *Eunicella stricta* (Octocorallia) do not respond to light either (Theodor, 1967). However, *Cyanea* planulae may be photonegative just prior to attachment, although Phillips (1972) remarks that the settlement of planulae of *Cyanea* on shell surfaces appears not to be affected by light; further work is required on the photic response, for this is affected not only by light intensity, but also by salinity and temperature (*e.g.*, Meadows and Campbell, 1972) and neither these parameters, nor others (see Crisp, 1974), are explored in the present study. Some illumination does seem to be necessary for optimal survival during the planula stage of *Cyanea,* perhaps by increasing attachment rate and thereby reducing the length of the free-swimming stage; madreporarian planulae attach more slowly in the absence of light (see Yonge, 1963, 1968). In the experiments reported, here, the response to light was weak and almost obscured by the response to direction of gravity.

The early planula of *C. capillata* are geopositive; Phillips (1972) notes the initial movement of *Cyanea* planulae to the bottom of culture dishes where they swim next to the substrate. The planula of *E. stricta* are possibly geopositive, also; their descent to the bottom is passive, however, and Theodor (1967) does not regard this to be a behavioral response. Whether the initial settlement of *Cyanea* planulae is an active response was not determined. Even if this is solely due to the effect of gravity, settlement to the bottom might be considered an active response, for *Cyanea* planulae are capable of swimming to maintain their position in the water column if the water contains large quantities of organic matter, living or dead. Perez (1920–21) records the activity of *Cyanea* planulae in his collecting buckets containing medusae, and this author has noted the same in those buckets left to stand unattended (medusae readily shed bits of tentacles and other tissues from the oral surface under these conditions).

It appears that a reversal of response to the direction of gravity is stimulated by anaerobic conditions; in effect, the planula becomes geonegative when exposed to a lowered oxygen concentration or associated factors. Additional work is required to determine whether this really is a response to a lowered concentration of oxygen or a response to an increase of carbon dioxide. The fact that planulae are more active in containers with organic matter than in those in which oxygen is replaced with nitrogen suggests that the latter is possible.

The planulae cultured in fresh, filtered sea water may create the stimulus for activity through their own metabolism, since they become densely concentrated in local patches at the bottoms of their containers. Populations of protozoa alter the oxygen tension (Fox, 1921) or carbon dioxide concentration (Jennings, 1906) in their immediate environment to which they respond by adjusting their distribution in this self-induced gradient. The ciliate-like planula might be expected to react to these conditions in a similar way.

Because the stimulus for increased activity of the planulae may be associated with the concentration of dissolved gases, it is significant that scyphozoan polyps are most frequently found in the field in areas of calm water (*e.g.,* Thiel, 1962) on hard substrate in close proximity to where sediments are fine and thus most conducive to anaerobic conditions at the water-sediment interface (*e.g.,* Hayes, 1964; Fenchel, 1969); under these conditions a gradient in dissolved gases (and other chemical characteristics) which extends a few millimeters above the sediment surface can become established (*e.g.,* Odum, 1971, pp. 343–344).

Planulae cultured in containers having a high concentration of organic matter attach rightside-up; this orientation at time of attachment is opposite of that occurring in most of the experiments. The data do not provide a direct answer for this paradox. This difference may be related to either or both the rate of change in the concentration of dissolved gases or to the extent of this change. Under extreme anaerobic conditions the planulae may not be capable of sustained activity, they sink, and attach rightside-up where they land. It was noted, above, that the planulae are not particularly active in the culture exposed to nitrogen; more attach rightside-up in this treatment than in either that which contains organic material or in the control. The possibility that mortality is highest in this treatment with nitrogen is also mentioned and is consistent with this interpretation. This suggests that attachment rightside-up may be, at least in part, a laboratory artifact which occurs under more extensive anaerobic conditions than would be expected in the field.

Response of planulae to physical characteristics of the substrate has been reported for Anthozoa (Yonge, 1963, 1968; Theodor, 1967) and for Hydrozoa (Williams, 1965; Teitelbaum, 1966) and is a well-recognized phenomenon among marine larvae in higher phyla (Reese, 1964; Newell, 1970; Meadows and Campbell, 1972; Crisp, 1974). Williams (1965) shows that planulae of *C. squamata* respond to substrate surface in two ways: first, they selectively settle in small depressions in a hard surface of uniform texture; and secondly, they are sensitive to surface roughness as measured by their rates of locomotion on different artificially roughened substrates. The first response is similar to that shown by the planulae of *Cyanea* which select grooves and depressions on the bottom of their container, although this is not a pre-attachment phenomenon in *Cyanea* as it is for *Clava.* The second response may be shown by the different numbers of *Cyanea* planulae encysting on the upper surface of smooth, roughened, and painted coverslips which suggests the capacity to discriminate among these three grades of roughness, but the rates of movement of the planulae resulting in this distribution of cysts was not measured. The possibility that this difference is due to an albedo response is lessened if it is recalled that planulae did not attach to the black slate bottom of the aquarium under identical illumination; however, response to a chemical difference on the painted coverslips cannot be ruled out. The response to substrate contour at time of attachment is shown in the relative distribution of planulae attaching to coverslips with grooves scratched on their lower surface; this may be the rugophilic response similar to that described by Crisp and Barnes (1954) for cyprid larvae, and since shown to be common among many marine larvae (Crisp, 1974). Likewise, planulae were observed to concentrate in the grooves made by pliers on split-shot when fastening them to coverslips. These laboratory data for *Cyanea* agree with the field observations of Cones and Haven (1969) who say that polyps of *C. quinquecirrha* are most numerous in depressions and along cracks in the surface of oyster shells.

The frequency with which planulae attach and metamorphose on the hyponeuston (Sars, 1841; Hargitt and Hargitt, 1910; personal observation), a smooth surface, may be another artifact associated with laboratory culture. The same phenomenon occurs with some sponge larvae and is similarly interpreted (see references in Fell, 1974).

Assuming, with appropriate reservation, that the events observed in the laboratory can be extrapolated to the field, these data suggest that under natural conditions attachment by planulae may be relatively rapid, being initiated not long after they encounter the conditions which occur at the water-sediment interface. The sometimes long and variable length of larval life observed during these experiments may be yet another laboratory artifact. The larvae of higher invertebrates can delay attachment in the absence of appropriate stimuli (*e.g.,* Newell, 1970; Crisp, 1974), and the same phenomenon has been observed by Phillips (1972) for *Cyanea* and by Cazaux (1958, 1961) for *H. echinata* (Hydrozoa).

It seems reasonable that the motile planula stage of *Cyanea* is of short duration. Long larval life is selectively advantageous for sedentary species in which this is a dispersal stage (*e.g.,* Thorson, 1950, 1961; Scheltema, 1967, 1971). In the Scyphozoa, unlike the situation in higher phyla with sedentary adults, the adult (pelagic medusa) is best suited for dispersal. The small, weakly swimming and primarily benthic planula might not be expected to serve this function effectively. Mackie (1974) questions the role of dispersal traditionally accorded the medusa, and emphasizes the selective advantage of maintaining a circumscribed distribution in shallow water where the sedentary phase of the cnidarian life history may be successfully completed. The rapid settlement to the bottom and subsequent attachment by the planula of *Cyanea* would fulfill this requirement. The probable limited movement of the planula on the bottom would involve solely the selection of a site for attachment, in a location presumably advantageous for the survival of the polyp.

Advantages for the polyp are uncertain. It might be that attachment on the underside of an object decreases the unfavorable consequences of sedimentation and overgrowth by other organisms (Cargo and Schultz, 1966). Also, in this position above the substrate the exposure to prolonged anaerobic conditions is reduced, and the array of potential food organisms possibly is increased. In addition, the process of strobilation and subsequent release of ephyrae may be more efficient from a pendant attitude.

The term *encystment,* applying to the planulae at time of attachment, implies a protective function for this stage between the free-swimming larva and the sedentary polyp. Preliminary observations suggest this is a possibility, but convincing data are not yet available and the observations of others conflict. MacMurrich (1891) reports almost universal encystment by planulae of *C. arctica* [= *C. capillata*], and personal observations, as well as those of Cargo and Schultz (1967) and Cargo (1975), indicate that encystment is the rule in *C. capillata;* similarly, Rees (1957) and Widersten (1968) report that encystment is a normal ontogenetic stage in the life history of *C. lamarcki* and *C. palmstruchi*, respectively. On the other hand, Hargitt (1902) and Hargitt and Hargitt (1910) suggest that encystment is facultative in *C. arctica* [= *C. capillata*] and is an adaptation to unfavorable conditions; the data of Hyde (1894) can be interpreted to support this conclusion. Resistance to summer temperatures by these cysts is implicit in Cargo (1975). The failure to mention encystment by *C. capillata* suggests that it does not occur in the material observed by Agassiz (1862), Perez (1920–21), or Widersten (1968); Sars (1841) implies that the cyst is an attachment disc. This observation by Sars (1841) suggests the possibility that the term *encystment,* applied to this stage in much of the literature, is inappropriate; Mergner (1971, p. 20) describes the formation of periderm-surrounded discs after the attachment and flattening by hydrozoan planulae, similar to observations of *Cyanea* made by this author and presumably others.

Russell (1970) notes that planula cysts of *Cyanea* have never been found in nature. Since the scyphistomae are capable of limited locomotion (Gilchrist, 1937; Chapman, 1966, 1968; Blanquet, 1972), the discovery of planula cysts of *Cyanea* in the field would not only provide clear evidence for the choice of attachment site (because they are fixed immovably to the substrate), but may also contribute to a better understanding of the phenomenon of encystment in this species.

Neither the effects of water movement nor the chemical influence of the substrate surface on attachment behavior of *Cyanea* planulae was studied. Either one or the other, or both, may affect the behavior of some larvae in the higher phyla (*e.g.*, Reese, 1964; Newell, 1970; Meadows and Campbell, 1972; Crisp, 1974), and similarly that of cnidarian planulae (Cazaux, 1958; 1961; Williams, 1965; Teitelbaum, 1966; Nishihira, 1965, 1967, 1968a, b, c; Theodor, 1967; Müller, 1969).

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## SUMMARY

The behavior of the planula of *Cyanea capillata* (Linneaus) is studied in the laboratory to determine if it responds to environmental cues in a way which may account for the distribution of its sedentary scyphistoma stage in the field.

The planulae react to gravity, water chemistry, surface texture, and possibly to light. They exhibit an initial geopositive response in well-aerated sea water, but become active and geonegative under conditions associated with local depletion of oxygen. Their attachment follows, and is usually upside-down on a horizontal surface. They attach with greatest frequency on substrates that are rough, and give evidence of discriminating among surfaces of different rugosity. The planulae possibly show a weak photonegative response at the time of attachment; confirmation of this photic response requires further work. These sequential responses shown in the laboratory result in an orientation at the time of attachment which is in agreement with the position usually occupied by the scyphistoma in the field.

The behavior of the planulae of *C. capillata* demonstrates a pattern of responses which may confirm, and extend to the Scyphozoa, the conclusion of others that a morphologically simple larva as found in the Cnidaria has the capacity to nonrandomly select specific substrate upon which to settle, a phenomenon well-documented for the more complex larval stages found in the higher Metazoa.

The attachment by planulae to the hyponeuston, their attachment rightside-up under anaerobic conditions in the laboratory, and the sometimes long duration of the motile phase of larval life are thought to be artifacts of laboratory confinement.

The adaptive significance of encystment at time of attachment is unresolved.

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